s1.m6.p14 **Identifying antagonists towards a G-protein coupled receptor using the structure of a hormone complexed to a neutralizing monoclonal antibody.** Galina Polekhina,[a] William J. McKinstry,[a] Hannelore Diefenbach-Jagger,[a] Patricia W. M. Ho,[a] Craig J. Morton,[a] Koh Sato,[b] Etsuro Onuma,[b] Matthew T. Gillespie,[a] T. John Martin[a] and Michael W. Parker[a], [a]*St. Vincent's Institute of Medical Research, Australia, and* [b]*Pharmaceutical Research Laboratory Chugai Pharmaceutical, Japan. E-mail: galinap@medstv.unimelb.edu.au*

Keywords: Receptor; Antibody; Antagonist

G-protein coupled receptors (GPCR) are the most common receptor class in humans controlling critical cell functions and are thus an important class of drug targets. They are notoriously difficult to over-express and crystallize, and to date only one high resolution structure (bacterial rhodopsin) is available. We present a promising new method for discovering GPCR agonists and antagonists using structures of GPCR ligands bound to antibodies. Parathyroid hormone related protein (PTHrP) was discovered as a hypercalcemia-causing factor of malignancy [1]. Under normal circumstances PTHrP plays a key role in regulating embryonic development of the skeleton and other tissues. PTHrP as well as parathyroid hormone (PTH) act upon the same GPCR, parathyroid hormone receptor (PTH-R). PTHrP has also been shown to be one of the main culprits of metastasis into bone of human breast cancer cells causing bone breakdown. We have crystallized and determined the structure of the complex between PTHrP and a neutralizing monoclonal antibody. The humanised version of this antibody is currently in clinical trials (Phase II) in Japan for treatment hypercalcemia, bone metastasis and cachexia [2]. The structure revealed that the residues of PTHrP involved in the interaction with the antibody are either strictly conserved among PTH and PTHrP sequences or have been implicated in the binding to the receptor. We therefore predicted that the binding pocket on the antibody resembles the receptor's. Using the structural data, we will computationally screen the large library of small "drug-like" molecules for the best fit into the PTHrP binding pocket of the antibody. The top hits will be tested in cellular assays for inhibition towards action of PTHrP on PTH-R. The structural biology results and those from the computational screen and cellular assays will be presented.

[1] Martin, T. J. (2002) *The Journal of Clinical Investigations*. **10**, 1399-1401.
[2] Sato, K. (1999) *The Seventh International Forum on Calcified Tissue and Bone Metabolism*. 32-37.

s1.m6.p15 **The mode of binding of isoniazid, an anti-tubercular drug, to arylamine n-acetyltransferase from *mycobacterium smegmatis*.** J. Sandy[a], S. Holton[b], M.E.M. Noble[b] and E. Sim[a]. [a]*Department of Pharmacology, University of Oxford, Oxford, OX1 3QT, UK;* [b]*Department of Molecular Biophysics, University of Oxford, Oxford , OX1 3QU, UK. E-mail: james.sandy@pharm.ox.ac.uk*

Keywords: Arylamine N-acetyltransferase; Isoniazid; Tuberculosis

Isoniazid is a frontline drug used in the treatment of tuberculosis (TB). Isoniazid is a prodrug, requiring activation in the mycobacterial cell by the catalase/peroxidase activity of the *katG* gene product. Tuberculosis kills 2 million people every year and the situation is getting worse due to the increase in prevalence of HIV/AIDS and the emergence of multidrug-resistant strains of TB.

Arylamine N-acetyltransferase (NAT) is a drug-metabolising enzyme (E.C. 2.1.3.5). The NAT enzyme is capable of acetylating and inactivating isoniazid, transferring an acetyl group from acetyl coenzyme A (AcCoA) onto the terminal nitrogen of the drug, which in its N-acetylated form is therapeutically inactive. The bacterium responsible for TB, *Mycobacterium tuberculosis*, contains and expresses the gene encoding the NAT protein [1]. It has been shown that isoniazid binds to the NAT protein from *Salmonella typhimurium* [2] and we report here the mode of binding of isoniazid in the NAT enzyme from *Mycobacterium smegmatis*, a close relative of the *M. tuberculosis* and *S. typhimurium* NAT enzymes.

The mode of binding of isoniazid to M. smegmatis NAT has been determined using data collected from two distinct crystal structures. This allows us to say with confidence that the mode of binding of isoniazid that we observe is not an artifact of the crystallization conditions used. We know that the NAT enzyme is active in mycobacterial cells and we propose that isoniazid binds to the NAT enzyme in these cells. NAT activity in M. tuberculosis is likely therefore to modulate the degree of activation of isoniazid by other enzymes within the mycobacterial cell. Determining the structure of NAT with isoniazid bound is likely to be useful in rational drug design for anti-tubercular therapy since NAT is a good drug target.

[1] Upton *et al.* (2001) *Molecular Microbiology* Vol. **42**, No. 2 : 309-317
[2] Delgoda *et al.* (2003) *BBA - General Subjects Vol*. **1620** : 8-14